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Cytomegalovirus membrane antigens

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SUMMARY

Cytomegalovirus (CMV) establishes a persistent latent infection in the human host. Healthy individuals rarely suffer from disease symptoms during primary infection nor do they experience symptomatic reactivation of the resident virus. In individuals with a deficient immune system primary infection or reactivation of the virus may lead to serious illness. In most individuals the (deficient) immune system manages to clear the host from an active CMV infection. So far a non-complicated therapy against CMV is not available.

Predominantly CMV is a cell-bound virus. Therefore the cell membrane is likely to contain the prime targets for elimination of the virus by the immune system. The aim in the present study was to characterize the specific membrane antigens which are recognized by the humoral immune system. An additional goal was to monitor humoral responses against these antigens.

In chapter 1 an introduction into CMV, CMV antigens, treatment and prophylaxis of CMV infection in renal transplant patients and immune responses against CMV is given. Special emphasis is given to the recent advances in the field of viral glycoproteins and the role of membrane antigens in specific defense mechanisms.

The isolation and characterization of monoclonal antibodies against viral membrane antigens is described in chapter 2. Partially purified plasma membrane vesicles prepared from infected fibroblasts were used to immunize mice. The selection of hybridomas was based on membrane fluorescence techniques. These experiments resulted in the isolation of only one type of monoclonal antibodies. The three polypeptides precipitated by this type of monoclonal antibodies were identified as members of the gC1 family of glycoproteins.

summary

The fact that only a limited number of viral antigens is recognized by human antibodies was revealed by studies described in chapter 3. Next to two gC polypeptides with molecular masses of 53-63 and 94-120 kDa, only one other polypeptide was precipitated from cell extracts prepared from CMV-infected surface-labelled fibroblasts by human antibodies. This 94 kD polypeptide was identified as a member of the gCIII family of glycoproteins. It was also shown that the number of surface polypeptides recognized before and after onset of active secondary CMV infection in renal transplant recipients remained the same.

Quantitation of the humoral responses against specific CMV antigens using immunoprecipitation methods or immunoblotting methods has proven to be difficult. Therefore two methods were developed to study immunological responses against single antigens in more detail. The method described in chapter 4 is based on the use of cloned fragments of the CMV genome. These fragments were expressed as fusion proteins in *Escherichia coli*. The fusion proteins proved to be useful as antigens in an enzyme-linked immunosorbent assay (ELISA). A drawback of this type of antigens is that the fusion proteins contain only a fragment of the CMV antigen and that this antigenic fragment is denatured during isolation of the fusion protein. The second method to detect antibody responses against single antigens was based on the capture of specific antigens by monoclonal antibodies. The specific antigen was bound to a monoclonal antibody that was immobilized in a microtiterplate, subsequently an ELISA was performed. The captured antigen is in its native state and thus contains linear as well as conformational antigenic epitopes. Chapter 5 contains a detailed description of the development of this assay.

The antigen capture immunoassay was used to study the responses against two different CMV antigens in healthy individuals, in patients with chronic renal failure and in renal transplant recipients suffering from primary or secondary CMV infections. These antigens were a gC polypeptide described in chapters 2 and 3, and the major immediate early antigen of CMV. The latter antigen is an intracellular antigen, but appears to be important in T-cell mediated cytotoxicity. In chapter 6 highly individual patterns of humoral responses against these two CMV antigens were demonstrated, moreover distinct differences in the kinetics of development of the two types of antibodies were demonstrated. In most patients suffering from a primary or a secondary CMV infection first detection of, or a significant rise of antibodies against the immediate early antigen preceded the first detection of, or a significant rise of, antibodies against the gC polypeptide.

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